

screened 12 OS and EWS cell lines for HER2 expression by FACS analysis and analyzed the ability of HER2-T cells to 1) proliferate, 2) produce immunostimulatory cytokines, and 3) kill HER2+ tumor cells in cytotoxicity assays upon exposure to HER2+ OS and EWS cell lines. The *in vivo* function was tested in a murine intraperitoneal xenograft model, which allows for serial imaging by bioluminescence with representative OS (LM7) and EWS (TC71) cell lines. Imaging results were confirmed by pathological examination. **Results:** 10 of 12 cell lines were HER2+ by FACS analysis. HER2-T cells recognized and killed all HER2+ OS and EWS cell lines in cytotoxicity assays, whereas HER2-negative tumor cells were not killed. Coculture of HER2-T cells with HER2+ tumor cell lines resulted in T-cell proliferation, and secretion of IFN- γ and IL-2 in a HER2-dependent manner. *In vivo*, HER2-T cells eradicated established intraperitoneal xenografts in 80% of animals harboring LM7 and 70% of those harboring TC71 tumors, resulting in long-term tumor free survival of treated animals. In contrast, delivery of non-transduced T cells did not change the tumor growth pattern. **Conclusions:** This study shows that HER2 is a target antigen for adoptive immunotherapy of OS and EWS. HER2-redirection T cells not only recognized and killed HER2+ tumor cells *ex vivo*, but also eradicated experimental xenografts *in vivo*. Hence, adoptive transfer of HER2-redirection T cells may represent a promising immunotherapeutic approach for OS and EWS.

246

OPTIMIZING IMMUNOTHERAPY IMMEDIATELY AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) TO EFFECTIVELY TREAT ESTABLISHED NEUROBLASTOMA

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Based on our previous success at inducing “protective” immunity to neuroblastoma immediately after syngeneic HSCT using a combination of adoptive immunotherapy and tumor vaccination, we hypothesized that a similar immunotherapeutic approach would be able to effectively treat mice with established tumors. Mice bearing established AGN2a (neuroblastoma) tumors were given lethal total body irradiation followed by HSCT consisting of bone marrow supplemented with 6×10^6 T cells. On days 2, 7, and 14 after HSCT, mice were vaccinated with irradiated AGN2a cells that had been genetically modified to express CD54, CD80, CD86, and CD137L. Bioluminescent imaging and caliper measurements were used to monitor tumor growth. Our best results – delayed tumor growth in all mice and 70% tumor-free survival – were seen when mice were given HSCT, T cells derived from tumor-vaccinated syngeneic donors (pre-sensitized T cells), and vaccination. Vaccination was an important component of the immunotherapy in these mice, as only 36% of mice given pre-sensitized T cells only (no vaccine) survived long-term. Several of the mice given pre-sensitized T cells without vaccination had late tumor recurrences, which were not observed in vaccinated mice. This may reflect the requirement of a vaccine-based approach for the induction or maintenance of long-term immune memory. Mice given HSCT, adoptive T cell transfer from naïve mice, and vaccination also showed delayed tumor growth, but tumor-free survival was lower (33%). Overall survival correlated with the frequency of IFN- γ -producing, tumor-reactive CD8+ cells in lymphoid tissues of treated mice. Moreover, we demonstrate that mice with regressing tumors had higher frequencies of tumor-reactive T cells in their lymphoid tissues than mice with progressing tumors. When specific immune cell subsets were depleted from the adoptively transferred T cells two important observations were made. First, CD4+ T cell depletion was found to initially benefit tumor-bearing hosts, but the generation of anti-tumor T cell memory was inhibited. Second, more specific depletion of CD4+25+ cells from the adoptively transferred T cells can result in better tumor immunity without decreasing anti-tumor T cell memory. In summary, our results suggest that a multi-faceted immunotherapeutic strategy including HSCT, T cell adoptive transfer and early post-transplant tumor vaccination can effectively treat mice with established neuroblastoma.

247

HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM-CELL SUPPORT VERSUS STANDARD-DOSE CHEMOTHERAPY FOR HIGH-RISK BREAST CANCER: META-ANALYSIS OF INDIVIDUAL PATIENT DATA FROM 15 RANDOMIZED ADJUVANT TRIALS

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Background: The role of adjuvant high-dose chemotherapy (HDC) with autologous hematopoietic stem-cell transplantation for primary breast cancer at high risk of recurrence (at least 4 involved axillary lymph nodes) remains ill-defined. Data from individual trials have limited power to show overall or subgroup benefit for this indication. **Methods:** Individual patient data from 15 known randomized trials (HDC vs standard-dose chemotherapy [SDC]) were merged into a single database. Disease-free survival (DFS) was defined as time from surgery to recurrence or death; breast cancer-specific survival (BCSS) was time from surgery to death from breast cancer or treatment-related toxicity; and overall survival (OS) was time from surgery to death. Cox proportional hazards regression was used to compare the effect of HDC vs SDC on DFS, BCSS, and OS adjusted for age, trial, hormone receptor (HmR) status (positive if either estrogen or progesterone receptor was positive), and menopausal (MP) status. Subgroup analyses were by age, HmR and MP status. **Results:** Median follow-up for 6,210 patients (3,118 HDC, 3,092 SDC) was 6 years (range, 0–15.3); median patient age was 46 years (range, 20–67). HmR status was positive in 46.8% of patients, negative in 23.7%, and unknown in 29.5%; 68.9% were premenopausal, 29.4% were postmenopausal, and 1.7% was unknown.

After adjusting for age, trial and MP, HDC was found to prolong DFS (hazard ratio [HR] 0.87; 95% confidence interval [CI] 0.81–0.94; $P = 0.0002$) but not BCSS (HR 0.93; 95% CI 0.85–1.02; $P = 0.12$) or OS (HR 0.95; 95% CI 0.87–1.03; $P = 0.18$). After adjusting for HmR in the subset for which that information was available, HDC was found to prolong DFS (HR 0.83; 95% CI 0.77–0.90; $P < 0.0001$) and had modest but significant benefits on BCSS (HR 0.88; CI 0.79–0.97; $P = 0.014$) and OS (HR 0.89; 95% CI 0.81–0.98; $P = 0.016$) compared to SDC. For BCSS and OS, none of age, MP or HmR had a significant interaction with treatment, yet there was a significant age by treatment interaction for DFS, in which HDC was better for younger rather than older patients. **Conclusions:** HDC as used in these 15 randomized studies prolongs DFS in adjuvant breast cancer. HDC has at most a modest benefit on BCSS and OS compared to SDC. Whether HDC has benefit in the context of contemporary taxane-based regimens and targeted therapies is unknown and may be resolved by future clinical trials.

248

KIR RECEPTOR-LIGAND INCOMPATIBILITY PREDICTS SUSCEPTIBILITY OF OSTEOSARCOMA TO NK-MEDIATED LYSIS

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Long-term survival for pediatric patients with high-risk solid tumors remains poor despite multi-modality treatment, and new approaches are needed. Although the success of KIR (Killer Immunoglobulin-Like Receptor) incompatible, haploidentical stem cell transplantation has been suggested in hematological malignancies in adults and children, it has not been thoroughly examined for pediatric solid tumors. In this study, we evaluated the potential for KIR-incompatible lysis of osteosarcoma cells, *in vitro*. We hypothesized that the killing of osteosarcoma cell targets could be predicted by the degree of KIR receptor/ligand mismatch. To test this hypothesis, healthy donor NK cells were isolated and KIR phenotype determined by flow cytometry. Consistent with previous studies, donor NK cells exhibited a high prevalence of all three